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Biomonitoring exposure to environmental tobacco smoke (ETS): A critical reappraisal

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1 **The most frequently used biomarkers for exposure to environmental tobacco smoke (ETS) are cotinine and thiocyanate in body fluids, carboxyhaemoglobin in red blood cells (COHb) and carbon monoxide in the expired air. Although not ideal, cotinine in blood, saliva or urine is an established biomarker for ETS exposure within the past 1–3 days. Comparison with cotinine concentrations in cigarette smokers reveals that passive smokers take up less than 1/100 of the nicotine dose of smokers.**

2 **Biomonitoring data available for the ETS-related exposure to genotoxic substances comprise uptake of benzene, polycyclic aromatic hydrocarbons (PAH), aromatic amines, tobacco-specific nitrosamines (TSNA), electrophilic compounds giving rise to urinary thioethers, mutagens causing urinary mutagenic activity and the formation of various DNA adducts. With the exception of TSNA, these biomarkers are related to chemicals occurring ubiquitously in the environment and in the food. As a consequence, the background levels in unexposed nonsmokers are high compared to the observed increases (if any) associated with ETS exposure.**

3 **Some markers of biological effects, which, by definition, are non-specific with regard to the underlying exposure, have also been investigated in relation to ETS exposure. These markers comprise cytogenetic effects, aryl hydrocarbon hydroxylase (AHH) induction, urinary hydroxyproline excretion and various factors indicative of cardiovascular risks. The available data suggest that passive smoking is associated with a small induction of placental AHH and also with effects on cardiovascular risk markers. The latter findings in particular may be confounded by other risk factors, which have been observed to be more frequent in passive smokers than in unexposed nonsmokers.**

Keywords: biomonitoring; environmental tobacco smoke; cotinine; thiocyanate; carbon monoxide; benzene

Introduction

Quantitative risk estimates for ETS-exposed nonsmokers published by various authorities rely almost exclusively on data from epidemiological studies.[1] It is the purpose of this review to summarize available data on the internal dose of tobacco smoke constituents and their possible effects in nonsmokers, exposed to ETS under real-life conditions. We feel that biological monitoring with nonsmokers exposed to ETS in their normal environment can provide objective data in this controversial field of research.

Surrogate biomarkers for ETS exposure

Surrogate biomarkers for ETS exposure are markers related to substances in tobacco smoke, which are assumed not to be implicated in toxicologically relevant processes, e.g. cancer, cardiovascular diseases or respiratory dysfunctions. The concentrations of precursors of surrogate biomarkers in ETS should be directly proportional to those of toxicologically relevant constituents in ETS. Suitable biomarkers should be specific for the exposure, their biological half-life should be long enough and the analytical method should be specific, precise, and not too labourious and expensive.[2] Strictly, these criteria are not met by any of the most commonly used surrogate biomarkers such as carboxyhaemoglobin or carbon monoxide in the exhalate, as well as cotinine and thiocyanate in body fluids (Table 1).

Carboxyhaemoglobin (COHb) and exhaled CO (COex)

Carbon monoxide (CO) is generated during all incomplete combustions of organic materials (for example cooking, heating or vehicle exhaust). In addition, CO is endogenously formed in mammals during catabolism of haem moieties.[3] Therefore, the biomarkers COHb and COex are by no means specific for tobacco smoke exposure. Normal or

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Table 1 Surrogate biomarkers for ETS exposure[a]

Biomarker	Precursor in ETS	Other sources	Significant association with the extent of real-life ETS exposure[b]
COHb, COex	CO	Incomplete combustions, endogenous formation	No[109,110]
Thiocyanate in body fluids	HCN	Diet	No[1,3,112]
Cotinine in body fluids	Nicotine	Diet(?)	Yes[12,112,113]

[a]Abbreviations: COHb: carboxyhaemoglobin; COex: carbon monoxide in exhaled air. [b]Literature cited is not comprehensive; for a review, see Benowitz.[16]

only marginally increased levels of COHb or COex were observed in passive smokers as compared to nonsmokers. This is compatible with the finding that the ETS-related increase of CO indoors amounts to only 0.5 – 1 p.p.m.[4]

Thiocyanate in body fluids

Thiocyanate is the detoxification product of cyanide with a half-life of 10 – 14 days.[3] The precursor of thiocyanate in tobacco smoke is hydrogen cyanide. Hydrogen cyanide levels in mainstream smoke (MS) of cigarettes vary between 300 and 550 μg/cigarette and the ratio of sidestream smoke (SS) to MS is 0.19 – 0.37.[6] In ETS, hydrogen cyanide occurs almost exclusively in the gaseous phase. Other sources for the intake of cyanide are almonds, pulses and maize. Cyanide is also formed in the colon by bacteria. Preformed thiocyanates are found in cabbage, turnips, mustard and cow milk.[3] Therefore, thiocyanate in body fluids is not specific for exposure to tobacco smoke. Although thiocyanate can be easily measured, and its long biological half-life prevents large fluctuations in body fluids, it is not specific enough to be suitable as a biomarker for ETS exposure under real-life conditions.

Cotinine in body fluids

Cotinine is the main metabolite of nicotine, the principal alkaloid of the tobacco plant. The nicotine yield of cigarette MS is about 1 mg, and the SS/MS ratio amounts to 2.6 – 3.3.[6] Nicotine occurs almost completely in the particulate phase of mainstream smoke and in the gaseous phase of ETS.[7] Average nicotine concentrations in indoor environments where smoking occurs are usually in the range of 1 – 10 μg/m³.[8,9] In addition, some *solanaceae*-derived food items such as tomatoes, potatoes and egg plants as well as tea contain small amounts of nicotine.[10] The biological half-life of cotinine is considerably longer than that of nicotine (16 – 20 h *vs* 1 h).[11] Therefore, cotinine is a much more reliable biomarker for active smoking, as well as ETS exposure, than is nicotine. In almost all studies, a statistically significant difference in cotinine levels between ETS-exposed and nonexposed nonsmokers was observed (Table 1). In addition, a significant relationship between the cotinine concentration in body fluids and the extent (frequency, duration and intensity) of ETS exposure was found. ETS exposure at home was often found to be more important than ETS exposure at the workplace or other places.[9,12] The ratio between the cotinine concentrations in ETS-exposed nonsmokers and smokers is usually < 1/100. However, there are general limitations for comparisons of active smoking with ETS exposure.[13] A major difference is the fact that cotinine in smokers is an indicator of the nicotine uptake with the particulate matter of mainstream smoke, whereas cotinine in nonsmokers indicates the exposure to the ETS gaseous phase.[14] The longer biological half-life of cotinine in nonsmokers as compared to smokers would lead to an overestimation of the exposure in passive smokers relative to smokers.[15] However, other researchers found similar pharmacokinetics of nicotine and cotinine in smokers and nonsmokers.[16] On the other hand, the significantly shorter half-life of airborne nicotine compared to other ETS components could lead to an underestimation of ETS exposure when based on cotinine concentrations in body fluids.[17] Airborne nicotine shows a high degree of adsorption to surfaces in indoor environments, which is responsible for the high decay rates of nicotine. Adsorbed nicotine can be released into the air so that nicotine, but not other ETS constituents, are measurable in environments in the absence of smoking.[18] This, together with possible dietary nicotine intake and transdermal nicotine absorption from nicotine-polluted surfaces might lead to an overestimation of cotinine-based ETS exposure.

Biomarkers for potentially genotoxic compounds related to ETS exposure

In contrast to biomonitoring for surrogate markers, which can give only indirect evidence of the uptake of substances of toxicological relevance, biomonitoring for potentially genotoxic compounds directly reflects the internal dose of substances possibly implicated in the process of carcinogenesis. However, with the exception of tobacco-specific nitrosamines, the genotoxic substances found in tobacco smoke occur almost ubiquitously in the environment and are taken up from various sources. Hence, for evaluating the contribution of ETS exposure to the total body burden, it is important to assess the background levels of these biomarkers. ETS exposure-related biomonitoring data for specific genotoxic compounds such as benzene, polycyclic

aromatic hydrocarbons (PAH), aromatic amines and tobacco-specific nitrosamines (TSNA) as well as group-selective analyses for various DNA adducts, urinary thioethers and mutagenic acitivity are available (Table 2).

Benzene

Benzene occurs ubiquitously in the environment, with traffic exhaust being the most important source.[19] Average benzene concentrations in rural and urban environments of 1–10 and 10–20 $\mu g/m^3$, respectively, have been reported in Germany.[20,21] Cigarettes were found to emit 30–50 μg/cigarette in MS and 345–653 μg/cigarette of benzene in SS.[22] In a household survey including 230 homes in Germany, the median benzene concentrations were 6.9 $\mu g/m^3$ in households with nonsmokers and 9.3 $\mu g/m^3$ in homes with at least one smoker.[21] Corresponding levels reported for the USA were 7 and 10.5 $\mu g/m^3$, respectively.[24] Various biomarkers can be used for the biological monitoring of benzene exposure.[25] Determination of benzene in blood[26] and exhalate[27] is suitable for biomonitoring acute exposure. Due to the longer half-lives of 6–12 h, the urinary benzene metabolites *trans,trans*-muconic acid and phenylmercapturic acid should preferably be used in field studies.[28,29] However, *trans,trans*-muconic acid is also a metabolite of the food preservative sorbic acid, and thus may not be a specific biomarker for low-level environmental benzene exposure.[30] Investigations under real-life conditions showed no, or only a marginal contribution, of ETS exposure to the background of benzene in nonsmokers (Table 2). Analysis of variance in one study showed that at most 15 % of the variation is explained by ETS exposure.[4] This is in good agreement with an air monitoring survey in 49 homes, which apportioned 11% of indoor air benzene to ETS.[31] Taken together, the biomonitoring data show that ETS exposure is only a minor source of the total benzene burden.

Polycyclic aromatic hydrocarbons (PAH)

PAH are formed during incomplete combustion of organic materials and are ubiquitously distributed in the environment. The main source of intake of PAH is the diet. In particular, fried, grilled, smoked and cured foods as well as leafy vegetables contain PAH mostly in the upper p.p.b. range with benzo[a]pyrene occurring in the lower p.p.b. range.[32] It is estimated that >90 % of the total PAH body burden originates from the diet.[32,33] In the MS and SS of a cigarettes about 10 and 100 ng of benzo[a]pyrene, respectively, are emitted.[34] Indoor air concentrations of benzo[a]pyrene were found to

Table 2 Biomonitoring for exposure to genotoxic compounds[a]

Precursor in ETS	*Other sources*	*Biomarker*	*Significant increase after real-life ETS exposure*
Benzene	Traffic exhaust, combustion, fuels	Benzene in blood or exhalate	No[114]/Yes[9,115]
	Sorbic acid	t,t-MA in urine	
PAH	Combustion, diet, ambient air	DNA adducts in WBC	(Yes[45])[b]
		Albumin adducts	No[39]/Yes[38]
		1-Hydroxypyrene in urine	No[Scherer, in preparation]
4-ABP	Gas or kerosene burners, diesel exhaust	4-ABP-haemoglobin adducts	No[40,70]/Yes[44,117]
NNK	–	NNAL and NNAL-glucuronide in urine	(Yes[75])[c]
NNK, NNN	–	HBP-haemoglobin adducts	No[40]
Polycyclic aromatics	Diet, ambient air, endogenous	Bulky DNA adducts in WBC and placenta	No[64,65,67,68]/(Yes[66])[c]
NDMA, NNK, (NDEA)	Diet, ambient air, endogenous formation	3-Methyl-/3-Ethyl-adenine in urine	(No[74])[d]
?	Oxidative stress (exogenous and endogenous)	8-OHdG in placenta	No[68]
Carbonyls (acrolein)	Diet	Thioethers in urine	No[76]
Aromatic amines(?)	Diet	Mutagenic activity in urine	No[76–78]/Yes[79]

[a]Abbreviations: t,t-MA: trans,trans-muconic acid; PAH: polycyclic aromatic hydrocarbons; WBC: white blood cells; 4-ABP: 4-aminobiphenyl; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL: 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanol; NNN: *N*-nitrosonornicotine; HPB: 4-hydroxy-1-(3-pyridyl)-1-butanone; NDMA: *N*-nitrosodimethylamine, NDEA: *N*-nitrosodiethylamine; 8-OHdG: 8-hydroxy-2'-deoxyguanosine. [b]Significant correlation between score for ETS exposure and DNA adducts. (Only 4 of 21 samples had detectable adduct levels.) [c]After high experimental exposure to sidestream smoke for 3 h. [d]No systematic assessment of ETS exposure. [e]After high experimental exposure to ETS for 8 h.

be 5 ng/m³ in homes with smokers and 3 ng/m³ in homes with nonsmokers.[15] The ultimate carcinogenic metabolites of benzo[a]pyrene and other PAH can bind to DNA or proteins. The long life-time of erythrocytes (120 days) leads to the accumulation of haemoglobin adducts and makes them suitable biomarkers reflecting exposure during the last 4 months. Because of the shorter half-life of albumin (20–24 days), its adducts reflect more recent exposure. Protein adducts can be taken as an index of the biologically effective dose of a carcinogen. Urinary excretion or monohydroxy phenanthrenes and 1-hydroxypyrene are also used for biomonitoring PAH exposure by passive smokers.[16] In most investigations, no increase in PAH biomarkers was found after ETS exposure. The two studies[37,38] showing significant ETS-related increases (Table 2) remain doubtful in view of the fact that (a) other working groups found only a small, if any, difference in protein PAH adduct levels between smokers and nonsmokers[39–41], (b) even extremely high exposure to ETS did not increase the urinary excretion of PAH metabolites[26] and (c) the estimated intake of PAH suggested an overwhelming role of diet.[33] Summarizing the available evidence for PAH exposure by passive smoking, it can be stated that the high background exposure to PAH from other sources, particularly from diet, excludes biomonitoring of these substances from being of any value in ETS risk assessment.

Aromatic amines

Haemoglobin adducts of 4-aminobiphenyl (4-ABP) and 3-aminobiphenyl (3-ABP) have been used to determine exposure to these substances by tobacco smoke. MS yields of 4-ABP and 3-ABP were found to be 2.4–4.6 and 2.7–5.0 ng/cigarette, respectively.[42,43] SS was found to emit 143 and 132 ng/cigarette of 4-ABP and 3-ABP, respectively.[6] In an experimental room containing an ETS-related carbon monoxide concentration of 8 p.p.m., a level of about 5 ng/m³ of 4-ABP was measured (Grimmer, personal communication). As yet, no other sources for 4- and 3-ABP have been identified, apart from the fact that 4-ABP was used in the dye industry decades ago.[44] However, 3- and 4-nitrobiphenyl, which are emitted by kerosene heaters and gas burners,[45] as well as by diesel engines, form the same haemoglobin adduct as do 3- and 4- ABP and must, therefore, be considered confounding factors. Controversial results on an effect of ETS exposure on the 4-ABP haemoglobin adduct level were reported (Table 2). In the most extensive study,[46] no ETS-related increase was observed. Nonsmokers living in cities were found to have 4-ABP-haemoglobin adduct levels 10–30 pg/g higher than nonsmokers living in rural areas.[47,48] This suggests that sources other than ETS are also important.

Tobacco-specific nitrosamines (TSNA)

TSNA are mainly formed from the tobacco alkaloids nicotine, nornicotine, anabasine and anatabine during fermentation of tobacco leaves. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*-nitrosonornicotine (NNN) are the biologically most important TSNA.[49] MS yields of commercial cigarettes were reported to be 17–306 ng/cigarette for NNK and 34–675 ng/cigarette for NNN.[50] Corresponding SS yields were 180–671 ng/cigarette and 141–348 ng/cigarette, respectively.[50] Reported concentrations of TSNA in rooms where smoking occurs, range from 0.2 to 29.3 ng/m³ for NNK and 0.7–23 ng/m³ for NNN.[51–53] As biomarkers for NNK exposure, urinary NNAL, the corresponding alcohol of NNK, and its glucuronide as well as pyridyloxobutylated haemoglobin and DNA have been used.[54] DNA and haemoglobin adducts, which also indicate exposure to NNN, release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) upon hydrolysis. There is no systematic investigation on the influence of everyday ETS exposure on urinary excretion of NNAL and NNAL glucuronide. In an experimental study, Hecht *et al*,[55] exposed five nonsmokers for 3 h in a small chamber of 16 m³ air volume to the extremely high SS concentrations of up to 230 μg/m³ nicotine and up to 263 ng/m³ NNK. Under these conditions, the urinary excretion of free and conjugated NNAL increased from 31 ± 41 pmol/d before exposure to 127 ± 74 pmol/d after exposure. The authors state that NNK uptake in smokers is about 120 times higher than in nonsmokers exposed to sidestream smoke.[55] We found in four of nine nonsmokers detectable NNAL and NNAL glucuronide levels (detection limit about 5 pmol/l) with an average excretion rate of 41 ± 52 pmol/d.[56] These results are comparable to the findings with nonsmokers before experimental SS exposure in the study of Hecht *et al*,[55] and suggest that the ETS-related NNK exposure under real-life conditions amounts to about 1% of the NNK dose in smokers.

HPB-releasing haemoglobin adducts could be tobacco-specific biomarkers of chronic exposure to NNK and NNN. Mean adduct levels for smokers and nonsmokers were 80 and 29 fmol/g haemoglobin, respectively, as reported by the working group of Hecht[57] and 69 and 34 fmol/g haemoglobin as found by the working group of Richter.[47] There was a large overlap between smokers and nonsmokers in both studies. The small differences in the HPB-adducts found between smokers and nonsmokers are remarkable. According to the NNK and NNN levels in MS and ETS as well as the urinary NNAL excretion rates discussed above, smokers should have HPB adduct levels at least two orders of magnitude higher than ETS-exposed nonsmokers. The lack of such a large difference might be explained by induction of TSNA detoxification and/or inhibition of TSNA activation in smokers.

Evidence for the latter hypothesis is provided by a recent finding suggesting that nicotine in smoking doses can inhibit NNK activation in rats.[58] In pregnant women, however, no relationship between ETS exposure (either based on self-reported exposure or classified by urinary cotinine levels) and HPB-haemoglobin adduct levels was observed.[46] In theory, NNK may also be formed endogenously by nitrosation of nicotine. No evidence for nitrosation of nicotine or cotinine to the possible nitrosation product 4-(*N*-methylnitrosamino)-4-(3-pyridyl)butyric acid (iso-NNAC) was observed.[59] In addition, the nitrosoproline test, which reveals an increased endogenous nitrosation capacity of smokers,[60] did not show elevated nitrosation in nonsmokers experimentally exposed to high ETS doses.[61,62] The two biomarkers for TSNA exposure, namely urinary NNAL and the HPB-haemoglobin adducts, need further validation before conclusions on ETS-related TSNA doses and possibly implicated risks can be drawn.

Various DNA adducts

Cigarette smoke condensate (CSC), when dermally applied to mice, was found to form various, as yet unidentified DNA adducts detectable by the ^{32}P-postlabelling method.[63] In human biomonitoring studies, available tissues and cells are limited to leucocytes, oral mucosa, exfoliated epithelial bladder cells, bronchial lavage cells and placenta. We are aware of only a few investigations where experimental[64,65] or real-life exposure to ETS[66–68] has been considered causative for the formation of DNA adducts detectable by the ^{32}P-postlabelling method. In one study, it was suggested that passive smoking might lead to the formation of a tobacco smoke-related adduct with placental DNA.[66] However, the evidence was weak, since ETS exposure was only reported for those nonsmokers who had detectable levels of this adduct. In another study with systematic assessment of ETS exposure in pregnant women, no increase in placental DNA adducts in passive or active smokers was found.[67] No additional DNA adducts or increases in DNA adduct levels after ETS exposure were observed in peripheral monocytes,[64] lymphocytes[65] or white blood cells.[67]

In addition to NNK, *N*-nitrosodimethylamine (NDMA) is another methylating agent in tobacco smoke. MS and SS yields were reported to be 0.1–20 and 143–1040 ng/cigarette, respectively.[42] ETS concentrations of NDMA in rooms with moderate smoking were 20–50 ng/m^3.[69] *N*-Nitrosodiethylamine (NDEA), *N*-nitrosomethylethylamine (NMEA) and ethylhalogenides are potential ethylating agents in tobacco smoke. However, only trace amounts of these nitrosamines are detectable in tobacco smoke,[70] whereas about 1 μg of ethylchloride was found in MS.[71] In an experimental study, no increase in the urinary excretion of 3-methyladenine or 3-ethyladenine was observed in nonsmokers exposed to high ETS concentrations.[72]

The promutagenic DNA adduct 8-hydroxy-2'-deoxyguanosine (8-OHdG) formed by reactive oxygen species is an established biomarker for oxidative DNA damage.[73] *In vitro* experiments have shown that the MS of cigarettes can induce 8-OHdG adducts after metabolic activation, the gaseous phase being responsible for this effect.[74] In a study aimed at systematically assessing ETS exposure in pregnant women, no ETS exposure-related increases in placental 8-OHdG adduct levels were found.[75]

Electrophilic compounds

Electrophilic chemicals are considered potential toxicants, mutagens and/or carcinogens because they may covalently bind to cellular macromolecules such as proteins, RNA and DNA.[76] Conjugation of electrophiles to glutathione (GSH), either spontaneously or enzymatically by means of glutathione *S*-transferases (GST), in most cases indicates a detoxification process. The GSH adducts are further metabolized to form *S*-substituted *N*-acetyl-L-cysteine conjugates (thioethers, mercapturic acids), which are renally excreted. Urinary thioethers have been used as group-selective biomarkers for the exposure to electrophiles.[77] Passive smoking under real-life conditions did not lead to a measurable increase in urinary thioether excretion (Table 2).[78] In an experimental study under dietary control and exposure to high ETS concentrations over a period of 8 h, a significant increase in excretion as compared to sham exposure was observed.[65] This effect was found to be caused by exposure to the gaseou.. phase of ETS. As a specific thioether, 3-hydroxypropyl mercapturic acid was identified, which is probably related to acrolein exposure.[65,74]

Mutagens

The mutagenic properties of CSC in short-term tests after metabolic activation are well established.[42] Measurements of the urinary mutagenicity in nonsmokers experimentally exposed to high doses of ETS yielded controversial results.[14] The influence of real-life ETS exposure on urinary mutagenicity has been determined in five populations.[76–79] No significant influence of ETS exposure was found in all but one investigaton.[79] More extensive studies are needed in order to quantify the contribution of ETS exposure to urinary mutagenicity, which is primarily dominated by dietary factors.[80]

Markers for biological effects related to ETS exposure

By definition, biological effect markers are not specific for the underlying exposure but reflect

physiological or toxicological responses to a great number of factors. Therefore, it is extremely important to control for confounding factors when applying these markers in population studies. Data about various biological effect markers in relation to ETS exposure are summarized in Table 3.

Cytogenetic effects

Tobacco smoke and its condensate have been shown to induce various cytogenetic damages and effects such as chromosomal aberrations (CA), sister chromatid exchanges (SCE) and micronuclei (MN) *in vitro* and *in vivo*.[77,81] The influence of ETS exposure on cytogenetic parameters has been investigated in three limited studies[77,82,83] and one more extensive approach.[84] No effect of passive smoking was found, probably because these tests were not sensitive enough to detect effects arising from low exposures, such as passive smoking.

Aryl hydrocarbon hydroxylase (AHH) induction

It is well documented that cigarette smoking can induce, via the *Ah* receptor, enzymes involved in the activation (e.g. cytochrome P4501A1) and detoxification (glutathione S-transferase, UDP-glucuronyltransferase) of xenobiotics. Induction is particularly high in the placenta.[85] The role of exposure to ETS in AHH induction has been investigated in human placenta at the enzyme[86,87] and messenger RNA level.[88] The results suggest that passive smoking may exert an inducing effect in the placenta. Further investigations are needed to clarify this possible effect of ETS exposure. Whether induction of the AHH enzyme system may increase the risk after exposure to PAH or other chemicals which are activated by cytochrome P450 enzymes or whether induction may be protective, as suggested by Remmer,[47] is an open question.

Urinary hydroxyproline (HOP) excretion

Increased urinary excretion of HOP is an established biomarker for certain osteopathic destructions, some endocrinological disorders and severe burns.[90] Since HOP is a degradation product of lung collagen and elastin induced by exposure to nitrogen dioxide (NO_2), this marker has also been used to study the effect of low NO_2 exposures arising from environmental automobile exhausts as well as acitive and passive smoking.[91] The results reported in the literature on the effects of active and passive smoking on urinary HOP excretion are controversial: while Kasuga[91] found significantly increased levels of this marker in smokers and passive smokers, these findings were not confirmed by others.[92,93] In a recent study, no relationship between personal NO_2 and ETS exposure and urinary excretion of HOP or desmosine, a catabolic product of elastin, was found.[94]

Effect markers for cardiovascular diseases

The pathomechanism by which active or passive smoking might increase the risk of cardiovascular disease is not completely understood. Possible effects include reduced oxygen supply, endothelial injury, reduction in high-density lipoprotein (HDL) cholesterol, increase in low-density lipoprotein (LDL) cholesterol, increased LDL oxidation with subsequent foam cell formation, increased platelet activation and coagulation.[95–97] Physiological changes in parameters related to these effects can be used as biological effect markers. In a couple of studies, the influence of ETS exposure under experimental and real-life conditions on possible effect markers has been investigated (Table 3).

In a small ETS field study, no effect of ETS exposure on either urinary 2,3-dinorthromboxane B_2 or 2,6-dinor-6-ketoprostaglandin $F_{1\alpha}$ excretion was found.[98] Sinzinger and coworkers reported a significant reduction in platelet sensitivity to

Table 3 Monitoring of biological effects related to ETS exposure[a]

Biological effect or biomarker	*ETS component possibly implicated*	*Significant effect of ETS exposure under real-life conditions*
SCE, CA in lymphocytes	ETS particulate matter (?)	No[77,82–84]
AHH induction in placenta	PAH, others	Yes[86–88]
Hydroxyproline in urine	NO_2 (?)	No[92,93]/Yes[91]
Total cholesterol	?	No[109,118]/Yes[119]
HDL in plasma	?	No[109,118]/Yes[107,119]
LDL in plasma	?	No[109,118,119]
Platelet aggregation	?	Yes[102]
Fibrinogen in plasma	?	Yes[105]
Tx-M and PGI-M in urine	?	No[98]
Carotid wall thickness	?	Yes[103,104]

[a]Abbreviations: SCE: sister chromatid exchanges; CA: chromosomal aberrations; AHH: aryl hydrocarbon hydroxylase; PAH: polycyclic aromatic hydrocarbons; HDL: high-density lipoprotein; LDL: low-density lipoprotein; Tx-M: 2,3-dinorthromboxane B_2; PGI-M: 2,6-dinor-6-ketoprostaglandin $F_{1\alpha}$.

antiaggregatory prostacyclins after acute passive smoking.[99–101] However, these authors exposed nonsmokers to ETS from 30 cigarettes over 15 min in a 18 m³ chamber, stating that this exposure would 'resemble that in discos, restaurants and the like'.[99] Unfortunately, no concentrations of ETS components in the chamber were reported. According to our experience, this ETS exposure far outreaches the levels in real-life conditions. In addition, no sham exposed control group was investigated in order to exclude unspecific, possibly stress-related effects. Davis *et al*,[102] reported a reduced platelet aggregate ratio after passive smoking, which would reflect an increased formation of platelet aggregates. In addition, these authors found an increase in the counts of anuclear endothelial cell carcasses, suggesting endothelial damage after ETS exposure. These findings need confirmation with larger groups of subjects exposed under well defined experimental conditions.

In two large population studies, ETS exposure was found to significantly increase the carotid wall thickness[103,104] and the plasma fibrinogen concentration.[105] Although the observed changes are small and the biological significance in terms of cardiovascular risk is unclear, the effects are relatively large in relation to the effect observed in active smokers. Both studies claim to have controlled for the major cardiovascular risk factors such as age, body mass index, ethanol intake, hypertension, diabetes, and total fat intake. However, since it is well established that the passive smoking status can be associated with an unfavourable constellation of a great number of cardiovascular risk factors,[106] it seems doubtful whether statistical control of confounding factors can be complete.

Conclusions

During the past 10–20 years, a large amount of data on biomonitoring of ETS exposure has accumulated. Cotinine in body fluids of nonsmokers has proven to be an acceptable, although nonideal biomarker for assessing ETS exposure dating back 1–3 days. This marker has been and will be almost routinely used in field studies in addition to or for validation of self-reported exposure to ETS. Nonsmokers, chronically exposed to ETS under real-life conditions, show cotinine concentrations in blood, saliva and urine at least two orders of magnitude lower than those of current cigarette smokers. Although nicotine uptake by active and passive smoking differs in many aspects, this comparison gives a good estimate for the dose difference between smoking and ETS exposure. COHb and CO in the exhalate as well as thiocyanate in body fluids, which all are also used as biomarkers for ETS exposure, are less suitable due to their low specificity for tobacco smoke exposure. However, COHb and exhaled CO have proven to be useful biomarkers for acute tobacco smoke exposure under well defined experimental conditions.

Biomonitoring for exposure to ETS-related genotoxic compounds might provide more relevant information for risk assessment than do the surrogate biomarkers cotinine, COHb and thiocyanate. Data are available for exposure to benzene, PAH, aromatic amines and tobacco-specific nitrosamines. In addition, various DNA adducts in nucleated blood cells and placenta as well as urinary thioether excretion and mutagenic activity have been investigated in relation to ETS exposure. The results obtained are not conclusive, but suggest that every-day ETS exposure, if at all, only marginally increases the levels of these markers above background levels. Three major difficulties have to be considered when interpreting these data: (1) it is yet unknown which carcinogens in tobacco smoke are responsible for tumor induction in humans. As a consequence, it is not possible to focus the biological monitoring on the appropriate chemicals or classes of chemicals. (2) Population biomonitoring is limited to readily available body fluids (blood, urine, saliva), cells (blood cells, oral mucosa cells, exfoliated bladder cells) and tissues (skin, nails, hair, placenta). With respect to the most widely discussed cancer risk attributed to ETS exposure, namely lung cancer, these materials represent no target cells or tissues, but allow only the determination of surrogate biomarkers. (3) With the exception of TSNA, the biomarkers for exposure to genotoxic compounds applied today are not related to substances unique for tobacco smoke, but occur ubiquitously in the environment and in food. Therefore, the existing background exposure to these substances has to be considered when evaluating an ETS-related increase in risk. As discussed above, the most promising biomarkers for TSNA exposure, i.e. urinary NNAL and the TSNA-related haemoglobin adducts, need further validation before being applicable for the biomonitoring of ETS exposure.

ETS exposure was not found to exert cytogenetic effects such as SCE or CA in peripheral lymphocytes. However, these biomarkers may not be sensitive enough to indicate effects after low exposure, such as passive smoking.

Controversial results were reported on the effect of ETS exposure on urinary hydroxyproline (HOP) excretion. The findings of Kasuga,[93] who found a significant dose-related effect of ETS exposure on urinary HOP excretion, could not be confirmed by other investigators.[92–94] Further studies are needed to clear this discrepancy.

A small AHH-inducing effect of ETS exposure in human placenta was found. The biological significance of this finding is unclear, because induction

comprises most probably enzymes involved in both activating and detoxifying pathways of carcinogens. It would be of interest to investigate ETS exposure-related enzyme induction in other relevant organ systems (e.g. the lung).

Significant, unfavourable effects of ETS exposure were reported for some biomarkers, which are regarded to be indicative for the development of cardiovascular diseases. These include total serum cholesterol, HDL, plasma fibrinogen, platelet aggregation and carotid wall thickness. The extent of these effects was surprisingly high as compared to that observed with smokers. To explain this discrepancy, it is hypothesized that nonsmokers are more sensitive to some tobacco smoke components than are smokers.[101,102] An alternative explanation would be that passive smokers differ from nonsmokers not only in their exposure to ETS, but also in a series of other cardiovascular risk factors.[100] The alleged detrimental effects of ETS exposure on the cardiovascular system are an important issue for future research.

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